



US006387888B1

(12) **United States Patent**  
**Mincheff et al.**

(10) **Patent No.:** **US 6,387,888 B1**  
(45) **Date of Patent:** **May 14, 2002**

(54) **IMMUNOTHERAPY OF CANCER THROUGH  
EXPRESSION OF TRUNCATED TUMOR OR  
TUMOR-ASSOCIATED ANTIGEN**

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(\* ) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/164,034**

(22) Filed: **Sep. 30, 1998**

(51) **Int. Cl.**<sup>7</sup> ..... **A61K 48/00**; C12N 15/63;  
C12N 15/85; C07H 21/02

(52) **U.S. Cl.** ..... **514/44**; 435/320.1; 435/325;  
536/23.1; 536/23.5

(58) **Field of Search** ..... 514/44; 424/93.1

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(57) **ABSTRACT**

DNA constructs for truncated forms of cancer-specific or  
cancer associated antigens are included in plasmid or viral  
expression vectors. The rationale to use constructs for trun-  
cated and not for full-size molecules is to eliminate side  
effects (toxicity, signal transduction etc.) arising from  
expressed proteins and/or, in cases where such molecules are  
expressed on the membrane, secreted, or released in the  
extracellular environment, to prevent formation of antibod-  
ies against them. The extracellular portion of the human  
prostate specific membrane specific antigen (XC-PSMA)  
has been cloned. Patients were treated either by injection of  
DNA coding for XC-PSMA in a mammalian expression  
vector under the CMV promoter or/and by a replication-  
defective adenoviral vector (Ad5) that contains an expression  
cassette for the XC-PSMA. In a third method dendritic cells  
are isolated from a patient and are treated by exposure to the  
plasmid or adenovirus used in the previous two treatments.  
The dendritic cells are then injected into the patient. In some  
patients, the progression of metastatic prostate cancer is  
retarded or stopped.

**30 Claims, No Drawings**

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**IMMUNOTHERAPY OF CANCER THROUGH  
EXPRESSION OF TRUNCATED TUMOR OR  
TUMOR-ASSOCIATED ANTIGEN**

**CROSS-REFERENCE TO RELATED  
APPLICATIONS**

Not Applicable.

**STATEMENT REGARDING FEDERALLY  
SPONSORED RESEARCH OR DEVELOPMENT**

Not Applicable.

**BACKGROUND OF THE INVENTION**

**(1) Field of the Invention**

The present invention relates to compositions and methods for immunotherapy of human cancer patients.

**(2) Description of Related Art Including Information Disclosed Under 37 CFR 1.97 and 37 CFR 1.98.**

All normal human nucleated cells express on their membrane small protein fragments derived from de novo protein synthesis. These so-called peptides are associated with the major histocompatibility complex (MHC) class I molecules and form the antigens which are recognized by CD8 cytotoxic T-lymphocytes (CTLs). Such recognition is important for the elimination of virally infected cells, of tumor cells, or of cells that contain intracellular parasites. For this to occur potentially antigen-reactive T cells need to be "pre-educated" by recognizing the antigen in question on the membrane of professional antigen-presenting cells (dendritic cells) (APCs, DCs) which, in addition to the antigen, provide co-stimulatory "maturation" signals to the T cells. In the absence of such signals the T cells become paralyzed and tolerant to the antigens in question.

Tumor cells, which are not professional APCs, do not stimulate CTL generation and are not rejected by the immune system. For the generation of an immune response against a tumor the tumor antigen(s) need(s) to be expressed by professional APCs. This presentation has been accomplished by in vitro exposure of dendritic cells to tumor lysates that presumably contain tumor antigens, to purified tumor antigens or, to peptides derived from such antigens.

Another possibility to achieve expression of antigen-derived peptides is by introducing into dendritic cell desoxy—(DNA) or ribonucleic acid (RNA) that encodes the antigen of interest. Cells transfected with the plasmid DNA transiently synthesize the protein and the peptides that are obtained during the synthesis are then expressed in association with MHC. For example, patient's cells grown in vitro are transfected with plasmids, containing the DNA, or with the RNA of interest or infected with a recombinant viral vector that contains the DNA or RNA, and then returned to the patient. Another possibility is to directly immunize the patient with the plasmid ("nude" DNA immunization) or with the recombinant viral vector.

A major problem with this technique comes from the possible adverse effects of the expressed products on the patient's health or on cell viability. Since the function of these tumor-associated or tissue-specific antigens is largely unknown, their synthesis and release by patient's cells in vivo may lead to serious side effects. Furthermore, in cases where dendritic cells are transfected in vitro, expression of a functional protein may alter dendritic cell viability, change their migration pattern or their ability to provide co-stimulation to T cells.

The present invention discloses the idea for the introduction of specific changes in the DNA or RNA encoding the

antigen in question as a way of solving this problem. Such changes result in the expression of functionally inactive products without affecting the efficiency of transcription and translation of the DNA, the translation of the RNA, or the generation of antigenic peptides. Specifically the present invention discloses the development of a DNA, which leads to expression of a truncated form of the human prostate specific membrane antigen (PSMA). In particular, we have developed a DNA construct with deletions of the membrane and the intracellular portions of the human PSMA. The resulting DNA encoding the extracellular portion of the PSMA (XC-PSMA) has been incorporated in mammalian expression vectors. PSMA is a type II protein, it lacks a hydrophobic signal sequence and therefore is not secreted by the cell that produces it. Since our construct lacks membrane and cytoplasmic sequences, the resulting protein is not expressed on the membrane, therefore does not transduce signals and is not released from the membrane. Cells transfected with the XC-PSMA plasmid retain viability and express PSMA-derived peptides.

Furthermore, since the synthesized protein is not released but remains confined to the intracellular milieu, there is no production of antibodies directed against the protein and the immune response remains strictly cell-mediated. The exquisite engagement of cell-mediated immunity against a particular antigen is very important especially in cases where the target antigen of interest is expressed on normal tissues that are anatomically sequestered in immunoprivileged sites such as the eye, brain, testis etc. Those tissues are inaccessible to cell mediated injury, but readily damaged by antibodies. Immunotherapy based on eliciting cellular responses to differentiation (tyrosinase; gp100; TRP1; TRP2; MART-1/Melan-A; membrane-associated mucin, MUC-1 mucin) or normal tissue-specific (PSMA, PSA) antigens constitute an example where the production of antibodies against the target must not occur.

In the first method of treating of prostate cancer patients, the plasmid is injected intradermally. In a second method of treatment, the plasmid is incorporated into the genome of a replication-deficient adenovirus, which is injected intradermally into a patient. In a third method of treatment, CD14+ monocyte cells of a prostate cancer patient are isolated and matured into dendritic cells (DC) and transfected with either the plasmid or the adenovirus of the first two methods. The DC are then stimulated to express MHCs and are infused back into the prostate cancer patient where they stimulate autologous T-cells. These stimulated T-cells then destroy both normal and malignant prostate cells.

The effect of all of these treatments is to either by-pass the normal tolerance for self-antigens or the tolerance to tumor antigens. This will enable the cytolysis of target normal and malignant prostate cells normally shielded from immune recognition. The destruction of normal prostate cells by this procedure is not detrimental to the patient. A malignant prostate (with its mixture of normal and malignant cells) customarily is destroyed through surgery or radiation in the conventional primary treatment for this disease.

U.S. Pat. No. 5,227,471 discloses the structure of the prostate-specific membrane antigen. A method for treating prostate cancer was disclosed which involves an antibody directed against the prostate-specific membrane antigen and a cytotoxic agent conjugated thereto. However, since the PSMA is expressed on normal brain cells, use of antibodies which can transverse through the blood-brain barrier and damage normal brain cells is not acceptable. Methods for imaging prostate cancer and an immunoassay for measuring the amount of prostate-specific membrane antigen also were disclosed.

U.S. Pat. No. 5,788,963 discloses the use of human dendritic cells to activate T cells for immuno-therapeutic response against primary and metastatic prostate cancer. Human dendritic cells are isolated and exposed to PSMA or peptides derived thereof in vitro. The PSMA or peptides are believed to exchange with peptides already bound to MHC molecules on the dendritic cells and thereby to be expressed in an immunogenic manner, enabling the DC to stimulate killer cells which then lyse prostate cells.

U.S. Pat. Nos. 5,227,471 and 5,788,963 are incorporated by reference herein.

The present invention differs from the prior art in that it causes the DC to present an antigen derived from prostate cancer cells on their surface through transfection with a plasmid or adenovirus. The transfection may occur in vivo using injected plasmid or adenovirus. Alternatively, the transfection may occur in vitro using purified DC precursor cells isolated from the prostate cancer patient's blood. If transfection is done in vitro, the transformed cells are injected into the patient. Transfected DC cells are superior to DC cells, which have been exposed to antigen in vitro because both their loading with antigen-derived peptide and their ability to stimulate killer cells are more efficient. In addition, in vivo transfection using a plasmid or adenovirus is less laborious and less expensive than in vitro methods. The use of transfected cells avoids the necessity of identifying peptides capable of binding to different HLA phenotypes, as is required in methods, which involve the addition of peptides to cells. Finally, the use of a DNA sequence that encodes a truncated molecule of the PSMA guarantees that the protein is not released by the transfected cells and no antibodies against the target protein that are potentially hazardous to normal brain tissue are produced. The methods of the present invention bypass the normal tolerance for self-antigens. This enables the cytolysis of target cells normally shielded from immune recognition.

Another application involves treatment of melanoma patients. Melanocyte differentiation antigen MART-1 is a common melanoma antigen recognized by many CTLs from melanoma patients. It represents a membrane protein of 118 aminoacids and a single transmembrane domain. Either DNA encoding for a truncated form with no transmembrane domain or a full-size protein with no leading sequence is included in a plasmid or viral expression vector and used for immunotherapy similar to the one described for prostate cancer patients.

Another application involves treatment of breast, ovary, uterine, prostate or lung cancer patients. Her-2/neu antigen is a member of the epidermal factor receptor family and is presumed to function as a growth receptor. It is a transmembrane protein and is expressed during fetal development and very weakly on normal cells as a single copy. Amplification of the gene and/or overexpression of the associated protein have been identified in many human cancers such as breast, ovary, uterus, stomach, prostate and lung. DNA encoding for a truncated form of the Her-2/neu protein lacking the transmembrane portion and the leading sequence is constructed and included in a plasmid or viral vector(s) and used for in vitro or in vivo modification of patient dendritic cells and for immunotherapy.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention discloses the idea for the construction of genetically modified forms of polynucleotides encoding either tissue-specific or tumor antigens and for the use of

such constructs for immunotherapy of primary or metastatic cancer. The genetic modification of the constructs leads to expression of either functionally inactive products or prevents functionally active molecules from being secreted or expressed on the membrane of transfected cells. Such genetic modifications, however, do not affect the antigenicity of the expressed protein, its primary structure or the generation of peptides available for binding to cell's MHC molecules. The polynucleotide may be either a DNA or RNA sequence. When the polynucleotide is DNA, it can also be a DNA sequence, which is itself non-replicating, but is inserted into a plasmid, and the plasmid further comprises a replicator. The DNA may be a sequence engineered so as not to integrate into the host cell genome. The polynucleotide sequences may code for a polypeptide which is either contained within the cells or secreted therefrom, or may comprise a sequence which directs the secretion of the peptide.

The DNA sequence may also include a promoter sequence. In one preferred embodiment, the DNA sequence includes a cell-specific promoter that permits substantial transcription of the DNA only in predetermined cells. The DNA may also code for a polymerase for transcribing the DNA, and may comprise recognition sites for the polymerase and the injectable preparation may include an initial quantity of the polymerase.

In many instances, it is preferred that the polynucleotide is translated for a limited period of time so that the polypeptide delivery is transitory. The polypeptide may advantageously be a therapeutic polypeptide, and may comprise an enzyme, a hormone, a lymphokine, a receptor, particularly a cell surface receptor, a regulatory protein, such as a growth factor or other regulatory agent, or any other protein or peptide that one desires to deliver to a cell in a living vertebrate and for which corresponding DNA or mRNA can be obtained.

In preferred embodiments, the polynucleotide is introduced into muscle tissue; in other embodiments the polynucleotide is incorporated into tissues of skin, brain, lung, liver, spleen or blood. The preparation is injected into the vertebrate by a variety of routes, which may be intradermally, subdermally, intrathecally, or intravenously, or it may be placed within cavities of the body. In a preferred embodiment, the polynucleotide is injected intramuscularly. In still other embodiments, the preparation comprising the polynucleotide is impressed into the skin. Transdermal administration is also contemplated, as is inhalation.

One example of this approach is the use of a DNA that encodes a truncated form of the human PSMA, which lacks the membrane, and cytoplasmic portions of the molecule. Such DNA has been included by us into mammalian expression vectors: a plasmid and a propagation deficient virus.

For treatment of prostate cancer patients, dendritic cells are prepared by transfection using either a plasmid or a recombinant replication-deficient adenovirus whose DNA includes DNA encoding a truncated fragment of the prostate specific membrane antigen. Dendritic cells may be transfected in vivo by injection of plasmid or recombinant replication-deficient adenovirus in the patient. Alternatively the DC may be transfected (infected) in vitro by treating isolated dendritic cell precursor cells with plasmid (or recombinant replication-deficient adenovirus). The dendritic cells are then injected into the patient.

Without wishing to be held to this theory, it is the inventors' belief that successful immunotherapy requires that the target antigen be presented by a DC simultaneously

to both the helper (CD4+ T cells) and the effector (CD8+ T cells) arms of the immune system. Recognition by CD4+ T cells requires that antigenic peptides be expressed in conjunction with class II MHC molecules on the DC surface. This can be achieved by in vivo or in vitro transfection of DC with plasmid or infection of DC with recombinant adenovirus, both of which carry the DNA for the extracellular fragment of PSMA.

PSMA expression is restricted to prostate epithelial cells (Horoszewicz J S, Kawinski E and Murphy G P. Monoclonal antibodies to a new antigenic marker in epithelial prostate cells and serum of prostate cancer patients. *Anticancer Res.* 7:927;1987) and human brain tissue (Luthi-Carter R, Barczak A K, Speno H, Coyle J T. Molecular characterization of human brain N-acetylated alpha-linked acidic dipeptidase (NAALADase). *J Pharmacol. Exp. Therap.* 286:1020;1998). The antigen is expressed on normal and neoplastic prostate cells in the prostate or in prostate tumor metastases. While other marker antigens for prostate carcinoma such as prostate acid phosphatase and the prostate specific antigen (PSA) are secreted antigens, PSMA is an integral membrane glycoprotein.

#### Cloning of extracellular fragment of PSMA

cDNA of PSMA extracellular fragment (2118 bp) was obtained using total mRNA from the prostate tumor cell line LNCaP.FGC—CRL 1740 (ATCC). A PSMA-specific 3'-primer was used for reverse transcription of mRNA which was performed using RT from avian myeloblastosis virus (Boehringer). The resulting cDNA was then amplified using High Fidelity PCR System (Boehringer), and the gel purified PCR product of expected length was cloned into pCR2.1 vector (Invitrogen). Two clones were selected and checked by DNA sequencing. The resulting construct contains a free of mutation extracellular portion of PSMA with NotI-Kozak sequence introduced by PCR at its 5' end and SfuI site at its 3' end.

Preparation of the mammalian expression vector for subcloning of the extracellular portion of PSMA.

The modified cloning vector pcDNA3.1 (Invitrogen) was used for subcloning. The vector provides human cytomegalovirus (CMV) immediate-early promoter/enhancer region permitting efficient, high-level expression of recombinant protein as well as 3' flanking region containing bovine growth hormone polyadenylation signal for efficient transcription termination and for increasing the half life of the mRNA in vivo. The neomycin resistance gene (NRG) was removed by digestion with NaeI endonuclease and ligation of the NRG-free fragment of the plasmid following gel purification.

Subcloning of the extracellular portion of PSMA into a mammalian expression vector.

The extracellular fragment of the PSMA was sub-cloned into a modified mammalian expression vector pcDNA3.1 by NotI-SfuI cloning sites. Both NotI and SfuI sites as well as Kozak sequence were introduced during the RT-PCR step of the cloning.

Deposit of modified mammalian expression vector pcDNA3.1.

The modified mammalian expression vector was deposited as Designation Number 203168 on Aug. 28, 1998 at the American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110.

Preparation of a replication-defective recombinant adenovirus Ad5-PSMA.

Ad5-PSMA recombinant adenovirus was prepared using the kit available from Quantum Biotechnology Inc. The

transfer vector was constructed by subcloning of the extracellular PSMA fragment into the plasmid pAdBN (Quantum). For this purpose the PSMA fragment was initially sub-cloned into an unmodified pCDNA3.1 vector (Invitrogen). The portion of the plasmid that contains the CMV promoter-PSMA fragment-PolyA signal was cut using BglII and SmaI restriction endonucleases. The resulting product was purified on an agarose gel and subcloned by BglII-EcoRV cloning sites into pAdBN transfer vector (Quantum Biotechnologies Inc., Montreal, Canada).

The transfer vector was linearized with ClaI and co-transfected with linearized Adenovirus DNA in 293A cells. The recombinant adenovirus was purified three times and clones that were positive for PSMA expression were selected by immunoblotting. The positive clone was amplified in 293 cells and then purified on two successive CsCl gradients. Finally the purified virus was dialyzed against PBS-5% sucrose.

The replication-defective recombinant adenovirus Ad5-PSMA. was deposited as Designation Number 203168 on Aug. 28, 1998 at the American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110.

In vitro experiments:

Gene Transfer Using Replication-Deficient Adenovirus.

Peripheral blood mononuclear cells (PBMC) from healthy anonymous donors were isolated from freshly drawn blood by density centrifugation on Ficoll-Paque at 468 g at 22° C. for 30 minutes. PBMC were resuspended in RPMI with 5% autologous serum (complete medium) culture medium at  $1 \times 10^6$  cells/ml and allowed to adhere onto 175 cm<sup>2</sup> polystyrene tissue culture flask. The flasks are incubated at 37° C. and shaken every 20 minutes during incubation. After 1 hrs at 37 C., non-adherent cells are removed and adherent cells are cultured in 30 ml medium containing 2 ng/ml granulocyte macrophage colony-stimulating factor (GM-CSF) obtained from Immunex, Seattle, Wash. and 4 ng/ml interleukin-4 (IL-4), obtained from Sigma. Cells are cultured for 5 days and then dendritic cells (DCs) harvested by centrifugation and used for experiments following verification by light microscopy examination and flow-cytometry.

DCs were infected with the virus at a multiplicity of infection (MOI) of 100. Infection experiment were carried out in polypropylene tubes to prevent the adherence of the cells. 50  $\mu$ l of viral suspension were inoculated into 50  $\mu$ l of cell suspension ( $1.5 \times 10^6$  cells) in complete RPMI-1640 medium containing 2% of autologous serum. After inoculation the cells were incubated 90 min at 37 C. in 5% CO<sub>2</sub> at the complete RPMI-1640 medium containing 2% of autologous serum, than washed three times and incubated in RPMI-1640 medium containing 10% of autologous serum for additional 24 h at 37 C. in 5% CO<sub>2</sub>. Expression of PSMA was tested by immunoblotting. Efficiency of infection of DC by the adenovirus in our experiments was 20% i.e. 20% of the DC were infected by the recombinant adenovirus.

In additional experiments DCs were obtained from HLA-A2+ patients, infected with adenovirus, and cultured with autologous T cells in CM for 3 days at 37° C. T cells were harvested at the end of the incubation, CD8+ T cells purified by negative depletion with anti-CD4 antibodies and complement and their cytotoxicity tested. The CD8+ T cells that had been stimulated by autologous DC infected with Ad5-PSMA were cytotoxic against the prostate tumor cell line LNCaP.FGC (also of the HLA A2+ phenotype), but not against Jurkat (T leukemia) or U937 (myelomonocytic cell line) cells. In comparison, freshly separated T cells showed no cytotoxicity against any of the three cell lines.

In vivo experiments:

Patient Treatment with Plasmid or Adenovirus

#### Study Design

One group of seven patients received three injections of XC PSMA-DNA vaccine (XC PSMA-CD86 plasmid) at the same dose (100 ug) at one-week intervals. Five patients (see table 1) received 10,000 IU Leukine (Immunex, Seattle, Wash. at the site of the plasmid application immediately or 24 and 48 hours after the immunization.

Additionally, two months later, these seven patients and a group of 2 new patients received three injections of a recombinant, replication-deficient adenoviral (Ad5-XC-PSMA) vaccine ( $5 \times 10^8$  PFUs per application) at one-week intervals.

Plasmid was injected intradermally between the first and second toe of the right leg or intramuscularly. The viral vaccine was administered intradermally in the navel area.

Constant monitoring of the clinical state and the vital signs was carried out for 2 hours after vaccination. If stable, the subject was allowed to leave the hospital. A brief follow-up visit occurred 24 (and 48 in the case of GM-CSF inoculation) hours later.

#### Inclusion Criteria

All patients signed an informed consent form before admission into the study. Data from monitoring visits were shared with the patients as the study proceeded, and the patients were reminded that they were free to withdraw from participation at any time. Only patients with advanced, hormone-resistant cancer or patients unable to find or administer hormone therapy were included into the study.

Patients with a history of another malignancy or with a serious active infection or with another illness were excluded from the study.

#### Monitoring Studies

Standard laboratory tests included CBC, urinalysis, liver enzymes, antinuclear antibodies, erythrocyte sedimentation rate, PSA. Each patient had a pelvic CAT scan, chest radiograph and a cardiograph on entry and on week 20 (week 10 for the 2 patients immunized with virus only). Safety was defined as lack of untoward clinical or laboratory events, with particular attention to local and systemic reactions, as well evidence of anti-nuclear antibody.

Additionally, analysis of CD/HLA DR<sup>+</sup>; CD4<sup>+</sup>; CD8<sup>+</sup>; CD3<sup>-</sup>/CD16<sup>+</sup>CD56<sup>+</sup>; CD3<sup>+</sup>; CD1<sup>+</sup>; CD25<sup>+</sup>; CD19<sup>+</sup> cells as well as CD4/CD8 ratio prior and following immunotherapy was performed by flow cytometry.

## Results

### Characteristics of Participants

Nine men, ages between 49 and 69 with advanced adenocarcinoma of the prostate, were included in the study. Three patients had a radical prostatectomy, 2 were in preparation for surgery, three were inoperable and one was operable but had other contraindications for surgery treatment. Two of the patients died due to advanced cancer disease.

### Safety monitoring results

The immunizations were well tolerated. No changes in vital signs occurred following injections or on follow-up visits.

Patients who received intradermal immunizations with plasmid had a minor DTH-like reactions 24 hours following the third immunization. Patients NN 8 and 9 developed a DTH reaction 24 hours following each administration of the recombinant adenovirus. Patients NN 1 through 7 had no DTH-like reactions 24 hrs after the first immunization with the viral vector, but developed DTH after the second and third immunization. All DTH-like local reactions were mild and resolved within 72-hrs post immunization.

Patient N 4 had a vesicular rash after the last viral immunization which was located on the back and which resolved in the next two days with no treatment.

Patient N 7 had a papular urticaria-like rash with small petechiae at the center which developed 24 hrs after the last plasmid immunization and which disappeared after the discontinuation of the antibiotic therapy he was receiving.

No significant changes occurred in erythrocyte sedimentation rate, CBC, serum creatinine or other blood chemistries, or urinalysis. Serum liver chemistry values remained within normal range in all subjects.

No significant changes in the analysis of CD/HLA DR<sup>+</sup>; CD4<sup>+</sup>; CD8<sup>+</sup>; CD3<sup>-</sup>/CD16<sup>+</sup>CD56<sup>+</sup>; CD3<sup>+</sup>; CD1<sup>+</sup>; CD25<sup>+</sup>; CD19<sup>+</sup> cells as well as CD4/CD8 ratio prior and following immunotherapy were detected.

No subject developed abnormal vital signs following injection, no significant increase in antinuclear antibodies titer were observed, and anti-DNA antibody was not detected.

For PSA values, CAT-scans, bone scintigraphy or lymph node metastases before and after immunization see tables 1 and 2.

Tables 1 and 2 show that in some patients the progression of metastatic prostate cancer was retarded or stopped.

TABLE 1

Patients were immunized initially three times at weekly intervals with PSMA plasmid. Two months later all patients but patient #7 received three additional immunizations at weekly intervals with the recombinant adenovirus.												
Patient #	Stage of disease	Type of immunization	Additional treatment	PSA(ng/ml)		CT scan		LN		Bone metastases	Side effects	
				before	after	before	after	before	after			
1	T <sub>4</sub> N <sub>x</sub> M <sub>2</sub> inoperable	3x plsmid i.d. 3 x Ad5PSMA	orchiectomy Casodex	6.3	-	+++	+++	-	+	++	++	exitus
2	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub> operable <sup>#</sup>	3x plsmid i.m. +GM-CSF 3 x Ad5PSMA	orchiectomy Androcur	14.38	0.28	+++	*	-	-	-	-	none
3	T <sub>4</sub> N <sub>x</sub> M <sub>0</sub> inoperable	3x plsmid i.d. 3 x Ad5PSMA	orchiectomy	33.0	0.04	+++	*	-	-	-	+	none
4	T <sub>4</sub> M <sub>x</sub> M <sub>2</sub> post BPH and TUR inoperable	3x plsmid i.d. +GM-CSF 3 x Ad5PSMA	orchiectomy (recently Flucinome)	1.11	3.8	++	++	-	-	+	++	none

TABLE 1-continued

Patients were immunized initially three times at weekly intervals with PSMA plasmid.  
Two months later all patients but patient #7 received three additional immunizations at weekly intervals with the recombinant adenovirus.

Patient #	Stage of disease	Type of immunization	Additional treatment	PSA(ng/ml)		CT scan		LN		Bone metastases	Side effects	
				before	after	before	after	before	after			
5	T <sub>2-3</sub> N <sub>0</sub> M <sub>0</sub> in preparation for surgery	3x plsmid i.d. +GM-CSF 3 x Ad5PSMA	MAB	3.01	0.05	++	*	-	-	-	-	none
6	T <sub>3-4</sub> N <sub>x</sub> M <sub>x</sub> post TUR	3x plsmid i.d. +GM-CSF 3 x Ad5PSMA	orchiectomy MAB	1.6	0.04	+++	*	-	-	-	-	none
7	T <sub>4</sub> N <sub>x</sub> M <sub>2</sub> post radical prostatectomy metastases	3x plsmid i.m. +GM-CSF	MAB	100		+++	++**	-		++		exitus skin rash ***

## Legend:

++; +++ increase in the size of the prostate gland or presence of metastatic tumor post radical prostatectomy (patient #7)

-; + lack (-) or presence of bone metastases or lymph node engagement

\* significant decrease in the size of the prostate gland.

\*\* - Patient #7. Lack of urine excretion from both urethers due to metastases prior to the immune therapy. Appearance of diuresis from the right kidney one month after the last immunization. Died due to mechanical illeus following blockade of the rectum and sigmoideum by metastases.

\*\*\* - Patient #7 had a mild skin rash 24 hrs post the third plasmid application which disappeared after discontinuation of the concurrent antibiotic therapy.

# - Patient #2 could not have surgery due to cardiovascular complications.

MAB- maximum androgen blockade with Zoladex, Casodex or Flucinome orchiectomy-always bilateral

TABLE 2

Patients who were immunized with recombinant adenovirus 3 times at weekly intervals.

Patient #	Stage of disease	Type of immunization	Additional treatment	PSA(ng/ml)		CT scan		LN		Bone metastases	Side effects	
				before	after	before	after	before	after			
8	T <sub>4</sub> N <sub>2</sub> M post radical prostatectomy metastases	3 x Ad5PSMA	MAB	32	NA	+++	NA	+++	NA	-	NA	none
9	T <sub>4</sub> NM <sub>2</sub> post radical prostatectomy metastases	3 x Ad5PSMA	MAB	4.47	NA	+++	NA	-	NA	+++	NA	none

-; ++; +++ lack (-) or presence of local tumor metastases, or lymph node engagement

MAB- maximum androgen blockade with Zoladex, Casodex or Flucinome

NA- not available

It will be apparent to those skilled in the art that the examples and embodiments described herein are by way of illustration and not of limitation, and that other examples

may be used without departing from the spirit and scope of the present invention, as set forth in the claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 1

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2133 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

-continued

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

(G) CELL TYPE: prostate cell line LNCaP

(B) CLONE: Molecular cloning of a complimentary DNA encoding a prostate-specific membrane antigen.

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Israeli, R.S., Powell, C.T., Fair, W.R.,  
Murphy, G.P.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATG AAA TCC TCC AAT GAA GCT ACT AAC ATT ACT CCA AAG CAT AAT	45
Met Lys Ser Ser Asn Glu Ala Thr Asn Ile Thr Pro Lys His Asn	
1 5 10 15	
ATG AAA GCA TTT TTG GAT GAA TTG AAA GCT GAG AAC ATC AAG AAG	90
Met Lys Ala Phe Leu Asp Glu Leu Lys Ala Glu Asn Ile Lys Lys	
20 25 30	
TTC TTA TAT AAT TTT ACA CAG ATA CCA CAT TTA GCA GGA ACA GAA	135
Phe Leu Tyr Asn Phe Thr Gln Ile Pro His Leu Ala Gly Thr Glu	
35 40 45	
CAA AAC TTT CAG CTT GCA AAG CAA ATT CAA TCC CAG TGG AAA GAA	180
Gln Asn Phe Gln Leu Ala Lys Gln Ile Gln Ser Gln Trp Lys Glu	
50 55 60	
TTT GGC CTG GAT TCT GTT GAG CTA GCA CAT TAT GAT GTC CTG TTG	225
Phe Gly Leu Asp Ser Val Glu Leu Ala His Tyr Asp Val Leu Leu	
65 70 75	
TCC TAC CCA AAT AAG ACT CAT CCC AAC TAC ATC TCA ATA ATT AAT	270
Ser Tyr Pro Asn Lys Thr His Pro Asn Tyr Ile Ser Ile Ile Asn	
80 85 90	
GAA GAT GGA AAT GAG ATT TTC AAC ACA TCA TTA TTT GAA CCA CCT	315
Glu Asp Gly Asn Glu Ile Phe Asn Thr Ser Leu Phe Glu Pro Pro	
95 100 105	
CCT CCA GGA TAT GAA AAT GTT TCG GAT ATT GTA CCA CCT TTC AGT	360
Pro Pro Gly Tyr Glu Asn Val Ser Asp Ile Val Pro Pro Phe Ser	
110 115 120	
GCT TTC TCT CCT CAA GGA ATG CCA GAG GGC GAT CTA GTG TAT GTT	405
Ala Phe Ser Pro Gln Gly Met Pro Glu Gly Asp Leu Val Tyr Val	
125 130 135	
AAC TAT GCA CGA ACT GAA GAC TTC TTT AAA TTG GAA CGG GAC ATG	450
Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys Leu Glu Arg Asp Met	
140 145 150	
AAA ATC AAT TGC TCT GGG AAA ATT GTA ATT GCC AGA TAT GGG AAA	495
Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala Arg Tyr Gly Lys	
155 160 165	
GTT TTC AGA GGA AAT AAG GTT AAA AAT GCC CAG CTG GCA GGG GCC	540
Val Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu Ala Gly Ala	
170 175 180	
AAA GGA GTC ATT CTC TAC TCC GAC CCT GCT GAC TAC TTT GCT CCT	585
Lys Gly Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala Pro	
185 190 195	
GGG GTG AAG TCC TAT CCA GAT GGT TGG AAT CTT CCT GGA GGT GGT	630
Gly Val Lys Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly Gly	
200 205 210	
GTC CAG CGT GGA AAT ATC CTA AAT CTG AAT GGT GCA GGA GAC CCT	675
Val Gln Arg Gly Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro	
215 220 225	
CTC ACA CCA GGT TAC CCA GCA AAT GAA TAT GCT TAT AGG CGT GGA	720



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Leu	Thr	Pro	Gly	Tyr	Pro	Ala	Asn	Glu	Tyr	Ala	Tyr	Arg	Arg	Gly		
				230					235					240		
ATT	GCA	GAG	GCT	GTT	GGT	CTT	CCA	AGT	ATT	CCT	GTT	CAT	CCA	ATT		765
Ile	Ala	Glu	Ala	Val	Gly	Leu	Pro	Ser	Ile	Pro	Val	His	Pro	Ile		
				245					250					255		
GGA	TAC	TAT	GAT	GCA	CAG	AAG	CTC	CTA	GAA	AAA	ATG	GGT	GGC	TCA		810
Gly	Tyr	Tyr	Asp	Ala	Gln	Lys	Leu	Leu	Glu	Lys	Met	Gly	Gly	Ser		
				260					265					270		
GCA	CCA	CCA	GAT	AGC	AGC	TGG	AGA	GGA	AGT	CTC	AAA	GTG	CCC	TAC		855
Ala	Pro	Pro	Asp	Ser	Ser	Trp	Arg	Gly	Ser	Leu	Lys	Val	Pro	Tyr		
				275					280					285		
AAT	GTT	GGA	CCT	GGC	TTT	ACT	GGA	AAC	TTT	TCT	ACA	CAA	AAA	GTC		900
Asn	Val	Gly	Pro	Gly	Phe	Thr	Gly	Asn	Phe	Ser	Thr	Gln	Lys	Val		
				290					295					300		
AAG	ATG	CAC	ATC	CAC	TCT	ACC	AAT	GAA	GTG	ACA	AGA	ATT	TAC	AAT		945
Lys	Met	His	Ile	His	Ser	Thr	Asn	Glu	Val	Thr	Arg	Ile	Tyr	Asn		
				305					310					315		
GTG	ATA	GGT	ACT	CTC	AGA	GGA	GCA	GTG	GAA	CCA	GAC	AGA	TAT	GTC		990
Val	Ile	Gly	Thr	Leu	Arg	Gly	Ala	Val	Glu	Pro	Asp	Arg	Tyr	Val		
				320					325					330		
ATT	CTG	GGA	GGT	CAC	CGG	GAC	TCA	TGG	GTG	TTT	GGT	GGT	ATT	GAC		1035
Ile	Leu	Gly	Gly	His	Arg	Asp	Ser	Trp	Val	Phe	Gly	Gly	Ile	Asp		
				335					340					345		
CCT	CAG	AGT	GGA	GCA	GCT	GTT	GTT	CAT	GAA	ATT	GTG	AGG	AGC	TTT		1080
Pro	Gln	Ser	Gly	Ala	Ala	Val	Val	His	Glu	Ile	Val	Arg	Ser	Phe		
				350					355					360		
GGA	ACA	CTG	AAA	AAG	GAA	GGG	TGG	AGA	CCT	AGA	AGA	ACA	ATT	TTG		1125
Gly	Thr	Leu	Lys	Lys	Glu	Gly	Trp	Arg	Pro	Arg	Arg	Thr	Ile	Leu		
				365					370					375		
TTT	GCA	AGC	TGG	GAT	GCA	GAA	GAA	TTT	GGT	CTT	CTT	GGT	TCT	ACT		1170
Phe	Ala	Ser	Trp	Asp	Ala	Glu	Glu	Phe	Gly	Leu	Leu	Gly	Ser	Thr		
				380					385					390		
GAG	TGG	GCA	GAG	GAG	AAT	TCA	AGA	CTC	CTT	CAA	GAG	CGT	GGC	GTG		1215
Glu	Trp	Ala	Glu	Glu	Asn	Ser	Arg	Leu	Leu	Gln	Glu	Arg	Gly	Val		
				395					400					405		
GCT	TAT	ATT	AAT	GCT	GAC	TCA	TCT	ATA	GAA	GGA	AAC	TAC	ACT	CTG		1260
Ala	Tyr	Ile	Asn	Ala	Asp	Ser	Ser	Ile	Glu	Gly	Asn	Tyr	Thr	Leu		
				410					415					420		
AGA	GTT	GAT	TGT	ACA	CCG	CTG	ATG	TAC	AGC	TTG	GTA	CAC	AAC	CTA		1305
Arg	Val	Asp	Cys	Thr	Pro	Leu	Met	Tyr	Ser	Leu	Val	His	Asn	Leu		
				425					430					435		
ACA	AAA	GAG	CTG	AAA	AGC	CCT	GAT	GAA	GGC	TTT	GAA	GGC	AAA	TCT		1350
Thr	Lys	Glu	Leu	Lys	Ser	Pro	Asp	Glu	Gly	Phe	Glu	Gly	Lys	Ser		
				440					445					450		
CTT	TAT	GAA	AGT	TGG	ACT	AAA	AAA	AGT	CCT	TCC	CCA	GAG	TTC	AGT		1395
Leu	Tyr	Glu	Ser	Trp	Thr	Lys	Lys	Ser	Pro	Ser	Pro	Glu	Phe	Ser		
				455					460					465		
GGC	ATG	CCC	AGG	ATA	AGC	AAA	TTG	GGA	TCT	GGA	AAT	GAT	TTT	GAG		1440
Gly	Met	Pro	Arg	Ile	Ser	Lys	Leu	Gly	Ser	Gly	Asn	Asp	Phe	Glu		
				470					475					480		
GTG	TTC	TTC	CAA	CGA	CTT	GGA	ATT	GCT	TCA	GGC	AGA	GCA	CGG	TAT		1485
Val	Phe	Phe	Gln	Arg	Leu	Gly	Ile	Ala	Ser	Gly	Arg	Ala	Arg	Tyr		
				485					490					495		
ACT	AAA	AAT	TGG	GAA	ACA	AAC	AAA	TTC	AGC	GGC	TAT	CCA	CTG	TAT		1530
Thr	Lys	Asn	Trp	Glu	Thr	Asn	Lys	Phe	Ser	Gly	Tyr	Pro	Leu	Tyr		
				500					505					510		
CAC	AGT	GTC	TAT	GAA	ACA	TAT	GAG	TTG	GTG	GAA	AAG	TTT	TAT	GAT		1575
His	Ser	Val	Tyr	Glu	Thr	Tyr	Glu	Leu	Val	Glu	Lys	Phe	Tyr	Asp		
				515					520					525		

-continued

CCA ATG TTT AAA TAT CAC CTC ACT GTG GCC CAG GTT CGA GGA GGG Pro Met Phe Lys Tyr His Leu Thr Val Ala Gln Val Arg Gly Gly 530 535 540	1620
ATG GTG TTT GAG CTA GCC AAT TCC ATA GTG CTC CCT TTT GAT TGT Met Val Phe Glu Leu Ala Asn Ser Ile Val Leu Pro Phe Asp Cys 545 550 555	1665
CGA GAT TAT GCT GTA GTT TTA AGA AAG TAT GCT GAC AAA ATC TAC Arg Asp Tyr Ala Val Val Leu Arg Lys Tyr Ala Asp Lys Ile Tyr 560 565 570	1710
AGT ATT TCT ATG AAA CAT CCA CAG GAA ATG AAG ACA TAC AGT GTA Ser Ile Ser Met Lys His Pro Gln Glu Met Lys Thr Tyr Ser Val 575 580 585	1755
TCA TTT GAT TCA CTT TTT TCT GCA GTA AAG AAT TTT ACA GAA ATT Ser Phe Asp Ser Leu Phe Ser Ala Val Lys Asn Phe Thr Glu Ile 590 595 600	1800
GCT TCC AAG TTC AGT GAG AGA CTC CAG GAC TTT GAC AAA AGC AAC Ala Ser Lys Phe Ser Glu Arg Leu Gln Asp Phe Asp Lys Ser Asn 605 610 615	1845
CCA ATA GTA TTA AGA ATG ATG AAT GAT CAA CTC ATG TTT CTG GAA Pro Ile Val Leu Arg Met Met Asn Asp Gln Leu Met Phe Leu Glu 620 625 630	1890
AGA GCA TTT ATT GAT CCA TTA GGG TTA CCA GAC AGG CCT TTT TAT Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr 635 640 645	1935
AGG CAT GTC ATC TAT GCT CCA AGC AGC CAC AAC AAG TAT GCA GGG Arg His Val Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala Gly 650 655 660	1980
GAG TCA TTC CCA GGA ATT TAT GAT GCC CTG TTT GAT ATT GAA AGC Glu Ser Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser 665 670 675	2025
AAA GTG GAC CCT TCC AAG GCC TGG GGA GAA GTG AAG AGA CAG ATT Lys Val Asp Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln Ile 680 685 690	2070
TAT GTT GCA GCC TTC ACA GTG CAG GCA GCT GCA GAG ACT TTG AGT Tyr Val Ala Ala Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser 695 700 705	2115
GAA GTA GCC GGG CCC TAA Glu Val Ala Gly Pro 710	2133

We claim:

1. A method for treating prostate cancer in a subject comprising administering to a subject having prostate cancer an effective amount of a polynucleotide operably linked to a promoter, which polynucleotide encodes a truncated form of human prostate specific membrane antigen (PSMA) comprising the extracellular domain and lacking functional transmembrane and cytoplasmic domains; to thereby destroy malignant prostate cells in the subject and thereby treat the prostate cancer in the subject.
2. The method of claim 1, wherein the truncated form of human PSMA lacks the transmembrane and cytoplasmic domains.
3. The method of claim 2, wherein the truncated form of human PSMA consists of the extracellular domain of human PSMA comprising SEQ ID NO: 1.
4. The method of claim 1, wherein the polynucleotide is included in a plasmid.
5. The method of claim 4, wherein the plasmid consists of the plasmid having ATCC Accession No. 203168.
6. The method of claim 1, wherein the polynucleotide is included in a viral vector.

7. The method of claim 6, wherein the viral vector is an adenovirus.

8. The method of claim 7, wherein the adenovirus is a replication-deficient adenovirus.

9. The method of claim 1, further comprising waiting for an appropriate time after the administration, and administering to the subject a second dose of a polynucleotide operably linked to a promoter, which polynucleotide encodes a truncated form of human PSMA comprising the extracellular domain and lacking functional transmembrane and cytoplasmic domains.

10. The method of claim 4, further comprising waiting for an appropriate time after the administration, and administering to the subject a dose of a viral vector encoding a truncated form of human PSMA comprising the extracellular domain and lacking functional transmembrane and cytoplasmic domains.

11. The method of claim 10, wherein the plasmid and the viral vector encode a truncated form of human PSMA comprising SEQ ID NO: 1.

12. The method of claim 6, further comprising waiting for an appropriate time after the administration, and adminis-

tering to the subject a dose of a plasmid encoding a truncated form of human PSMA comprising the extracellular domain and lacking functional transmembrane and cytoplasmic domains.

13. The method of claim 12, wherein the viral vector and the plasmid encode a truncated form of PSMA comprising SEQ ID NO: 1.

14. The method of claim 9, further comprising waiting for an appropriate time after the administration of the second dose, and administering to the subject a third dose of a polynucleotide operable linked to a promoter, which polynucleotide encodes a truncated form of human PSMA comprising the extracellular domain and lacking functional transmembrane and cytoplasmic domains.

15. The method of claim 8, wherein the adenovirus consists of the adenovirus having ATCC Accession No. VR2631.

16. The method of claim 9, wherein the two doses are administered at about a one-week interval.

17. The method of claim 14, wherein the three doses are administered at about one-week intervals.

18. The method of claim 10, wherein the viral vector is administered about two months after administration of the plasmid.

19. The method of claim 12, wherein the plasmid is administered about two months after administration of the vector.

20. The method of claim 10, wherein two doses of a plasmid are administered to the subject before administering two doses of a viral vector, wherein the plasmid and the viral vector encode a truncated form of PSMA comprising the extracellular domain and lacking functional transmembrane and cytoplasmic domains.

21. The method of claim 20, wherein the two doses of plasmid are administered to the subject at about a one-week interval; the two doses of viral vector are administered to the subject at about a one-week interval; and the first dose of the viral vector is administered about two months after the second dose of the plasmid.

22. The method of claim 21, wherein the truncated form of PSMA is a truncated form of PSMA comprising SEQ ID NO: 1.

23. The method of claim 1, further comprising administering to the subject a polynucleotide encoding CD86.

24. The method of claim 23, wherein the polynucleotide encoding the truncated form of PSMA and the polynucleotide encoding CD86 are included in a single plasmid or vector.

25. The method of claim 1, wherein the polynucleotide is administered intradermally.

26. The method of claim 1, wherein the polynucleotide is administered intramuscularly.

27. The method of claim 1, wherein granulocyte-macrophage colony stimulating factor (GM-CSF) is administered to the subject.

28. The method of claim 20, further comprising administering GM-CSF to the subject after administering the plasmid.

29. The method of claim 20, further comprising administering GM-CSF to the subject simultaneously with the administration of the plasmid.

30. The method of claim 28, further comprising administering to the subject a polynucleotide encoding CD86.

\* \* \* \* \*